REMARKS

Claims 1, 3, 4, 33, 39 and 84-90 were pending in the instant application. Claims 2, 5-18, 20, 23-26, 28-32, 37-38 and 64-83 were previously canceled without prejudice as directed to a non-elected invention. Claims 19, 21-22, 27, 34-36, 40-63 and 91-108 are withdrawn from consideration as drawn to a non-elected invention. Claims 40-63 (Group II) are related to claims 1-39 and 67-68 (Group I) as product and process of use. It is the Applicant's understanding that, once the pending product claims are found allowable, any non-elected process claims (Group II, claims 40-63) will be rejoined and examined if such process claims include all of the limitations of the elected product claims (MPEP §821.04).

Claims 1, 3-4, 39 and 86 have been amended. Accordingly, claims 1, 3-4, 33, 39 and 84-90 will be pending after entry of the instant amendment. Applicant reserves the right to prosecute the claims as originally filed in this or a continuing application.

Support for the claim amendments and new claims can be found throughout the claims and specification as originally filed. No new matter has been added. In particular, support for the phrase "wherein the siRNA retains the ability to inhibit expression of the target mRNA by at least 30%" can be found in the specification at least at page 30, lines 25-28, at page 60 lines 27-31, in Example XIII, and in particular at page 88, line 21, through page 89, line 24, and in Figures 13A-D.

Elections/Restrictions

Applicants gratefully acknowledge the Examiner's reconsideration of the restriction between Groups I and IV as set forth in the Supplemental Restriction Requirement mailed July 12, 2006 (Paper No. 20060707) and agreement to examine Group IV (claims 85-90) together with Group I (claims 1, 3-4, 33, 39, 84 and 86-90).

Acknowledgement of the Withdrawal of Previous Rejections

Applicants gratefully acknowledge the withdrawal of: (a) the previous rejection of claims 1-4 and 33-37 under 35 U.S.C. § 102(b) as being anticipated by Agrawal *et al.*; and (b) the

previous rejection of claims 1-2 and 67-68 under 35 U.S.C. § 102(b) as being anticipated by Parrish *et al.*.

Rejection of Claim 86 under 35 USC § 112, Second Paragraph

The Examiner has rejected claim 86 under 35 U.S.C. §112, second paragraph, as being indefinite for recitation of "the cleavage site referencing the antisense strand." The Examiner alleges that "it is unclear what is the cleavage site of a siRNA and further it is unclear where the cleavage site in reference to the antisense strand is located." The Examiner further contends that there is no antecedent basis in claims 1, 84 and 85, from which claim 86 depends, for recitation of the limitation "cleavage site."

Applicants respectfully traverse with the Examiner's rejection on the grounds that the claims are clear and definite. Applicants respectfully refer the Examiner to page 14, lines 23-30 of the specification, where Applicants define the term "cleavage site" as "the residues, e.g., nucleotides, at which RISC* cleaves the target RNA, *e.g.*, near the center of the complementary portion of the target RNA, *e.g.*, about 8-12 nucleotides from the 5' end of the complementary portion of the target RNA." The specification further teaches that "upstream of the cleavage site with reference to the antisense strand" refers to residues... 5' to the cleavage site in the antisense strand" and that "downstream of the cleavage site with reference to the antisense strand" refers to residues... 3' to the cleavage site in the antisense strand" (page 15, line 27 through page 16, line 2). Applicants submit that based on the foregoing teachings in the specification, one of skill in the art would find the claims clear and definite. Further, Applicants respectfully point out that claim 86, as currently amended, recites that the siRNA of claims 1, 84 or 85 "further comprises a cleavage site for RISC." Accordingly, there is sufficient antecedent basis within claim 86 for recitation of the phrase "within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site." In view of the foregoing, Applicants respectfully request that the rejection of claim 86 for indefiniteness be reconsidered and withdrawn.

Rejection of Claims 1, 3-4, 33, 39 and 84-90 under 35 USC § 103(a)

The Examiner has rejected claims 1, 3-4, 33, 38 and 84-90 under 35 U.S.C. §103(a) as being obvious over Tuschl *et al.* (WO 22/44321) in view of Eckstein *et al.* (US 5,672,695) and Parrish *et al.* (2000 *Molecular Cell* 6:1077-1087). The Examiner alleges that Tuschl *et al.* teach "a siRNA, 19-25 nucleotides in length (see page 4, lines 1-4) wherein the siRNA comprise sugar or backbone modifications to increase in vivo stability and teach a preferred embodiment wherein the 2'-OH group is modified with a 2'-fluoro group, for example (see page 6, lines 3-6)." The Examiner acknowledges that Tuschl *et al.* "does not explicitly teach cytidine or uridine nucleotides in the antisense or sense strands having 2'-fluoro modifications nor specifically teach adenosine or guanosine nucleotides in the antisense or sense strands having 2' modifications."

The Examiner further relies on Eckstein *et al.* for allegedly teaching that "modification of 2' hydroxyl position of the ribose sugar enhances the stability of RNA molecules" and for teaching "preferred modifications of the cytidine and uridine with 2'-fluoro analogues (see column 4, lines 9-25)." The Examiner further relies on Parrish et al. for allegedly teaching that "different chemical modifiers at the 2' position enhance the molecules specificity" and for teaching "modification of the cytidine and uridine nucleotides with a 2' fluoro group as well ds RNA with either the sense or antisense strands unmodified are capable of RNA interference (see Figures 5 and 6)." The Examiner alleges that

> [i]t would have been obvious... to use the general conditions taught by Tuschl *et al.* for making 2'-modified siRNA to discover the optimal number and placement of 2'-sugar modifications in any siRNA molecule, such that the resulting siRNA molecule was endowed with maximum stability and functionality. Additionally, it would have been obvious...to incorporate known modifications, such as 2'-fluoro modifications of cytidine and uridine as taught by Eckstein *et al.* and Parrish *et al.*, to impart increased stability and functionality in any siRNA because as stated by Eckstein *et al.*... RNA has very low stability under physiological conditions and therefore modifications of RNA will provide therapeutic RNA with enhanced stability against chemical and enzymatic degradation.

The Examiner concludes that "the invention as a whole would have been prima facie obvious to one of skill in the art at the time the invention was made." Applicants respectfully traverse this

rejection. The cited references, alone and in combination, fail to teach or suggest each and every element of the present invention as recited in the claims amended herein.

Claim 1, as currently amended, is directed to a small interfering RNA (siRNA), comprising a sense strand and an antisense strand, wherein the antisense strand is complementary to the sense strand and has a sequence sufficiently complementary to a target mRNA sequence to direct target-specific RNA interference (RNAi), and wherein the ***antisense strand*** is modified by the substitution of ***each uridine with a 2'-fluoro uridine and each cytidine with a 2'-fluoro cytidine***, such that *in vivo* stability is enhanced as compared to a corresponding unmodified siRNA, and wherein the siRNA retains the ability to inhibit expression of the target mRNA by at least 30%. Claims 3-4, 33, 38 and 84-90 depend from claim 1. In particular, claim 84 specifies that the sense strand is unmodified. Claim 85 specifies that the ***sense strand and antisense strand*** are modified by the substitution of ***each uridine with a 2'-fluoro uridine and each cytidine with a 2'-fluoro cytidine.*** Claim 86 specifies that the ***antisense strand*** is further modified by the substitution of ***each adenosine located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand with a 2'-deoxy adenosine*** and the substitution of ***each guanosine located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand with a 2'-deoxy guanosine***. Claim 87 specifies that the antisense strand is further modified by the substitution of ***each adenosine with a 2'-deoxy adenosine*** and the substitution of ***each guanosine with a 2'-deoxy guanosine***.

The teachings of Tuschl *et al.* are directed to siRNA molecules useful for mediating RNA interference. Tuschl *et al.* teach that "the RNA molecule may contain at least one modified nucleotide analogue," such as "sugar- or backbone-modified ribonucleotides" (page 5, lines 16 and 23-24). Tuschl *et al.* teach that "[i]n preferred sugar-modified ribonucleotides the 2' OH group is replaced by a group selected from H, OR, R, halo, SH, SR, NH2, NHR, NR2 or CN, wherein... halo is F, Cl, Br or I" (page 5, line 31 through page 6, line 2). As acknowledged by the Examiner, Tuschl *et al.* fail to teach or suggest ***the specific sugar-modified ribonucleotides of 2'-fluoro uridine, 2'-fluoro cytidine, 2'-deoxy guanosine and 2'-deoxy adenosine,*** let alone the particular combinations and positions of these sugar-modified ribonucleotides within an siRNA molecule as required by the instant claims.

Moreover, Tuschl *et al.* ***teach away*** from the claimed invention. In particular, Tuschl *et al.* teach that "[t]he nucleotide analogues may be located at positions where the target-specific activity, *e.g.*, the RNAi mediating activity is not substantially effected, *e.g.*, ***in a region at the 5'-end and/or 3'end of the double-stranded RNA molecule***" (page 5, lines 16-20). Further, Tuschl *et al.* teach that "not all positions of a siRNA contribute equally to target recognition" and that "mismatches in the ***center of the siRNA duplex are most critical*** and essentially abolish target RNA cleavage" (page 4, lines 24-26). Thus, Tuschl *et al.* teach that nucleotide modifications are preferably positioned at the ends, and not at internal positions, of the double-stranded RNA molecule, as internal modifications may effect RNAi activity. Accordingly, one would not have been motivated, based on the teachings of Tuschl *et al.*, to substitute ***each uridine*** or ***each cytidine*** throughout either strand of the siRNA molecule with a nucleotide analogue, let alone substitute ***each uridine and each cytidine*** with nucleotide analogues. Moreover, one would not have been motivated to substitute, ***in addition***, ***each guanosine*** or ***each adenosine*** at internal positions (*e.g.*, "located within 2 nucleotides upstream and 3 nucleotides downstream of the cleavage site referencing the antisense strand") or throughout the antisense strand with a nucleotide analogue, let alone substitute ***each guanosine and each adenosine*** at internal positions or throughout the antisense strand. One would simply not have been motivated, based on the teachings of Tuschl *et al.*, to introduce nucleotide analogues at internal positions, especially within the antisense strand, of an siRNA molecule, since Tuschl *et al.* teach that internal modifications may effect RNAi activity.

The teachings of Eckstein *et al.* fail to make up for the deficiencies of Tuschl *et al.* The teachings of Eckstein *et al.* are generally directed to ***ribozymes*** having enhanced stability against chemical and enzymatical degradation by the incorporation of at least one modified nucleoside. In particular, Eckstein *et al.* teach that a modified nucleoside is one in which "the hydroxyl group at the 2'-position of the ribose sugar is replaced by a modifier group selected from halo, sulfyhdryl, azido, amino, mono-substituted amino and disubstituted amino groups" (column 2, lines 60-65). Eckstein *et al.* fail to teach or suggest the incorporation of ***any*** modified nucleoside into an ***siRNA molecule***. One of skill in the art would not have been motivated to introduce the modified nucleosides taught by Eckstein *et al.* into an siRNA since ribozymes and siRNAs are ***completely different molecules*** having completely different mechanisms of action.

Moreover, even if the motivation to extend the teachings of Eckstein *et al.* to an siRNA were to exist, (which it does not), the skilled artisan would have had no reasonable expectation of success in using the Eckstein methodology. The skilled artisan would have readily understood, at the time the instant application was filed, that the results obtained for introducing a modified nucleotide into a ***ribozyme*** cannot be extrapolated to an ***siRNA molecule*** with any reasonable expectation of success because the molecules operate through very different cellular mechanisms. In particular, the state of the art at the time of filing recognized that a ribozyme is a self-cleaving enzyme. In contrast, RNAi was recognized to involve the assembly of the RNA molecule with protein components to form a nuclease complex, RNAi-Inducing Silencing Complex (RISC), that RISC utilizes an active mechanism to search for the homologous mRNA target and ultimately mediates cleavage and degradation of the mRNA target. Given the distinct mechanism of RNAi as compared to that of a ribozyme, the skilled artisan would not, based on the current state of the art and the teachings of the cited references, have had any reasonable expectation of success in making and using an siRNA as claimed. One skilled in the art would recognize that specific modified nucleosides tolerated in a ribozyme or in an siRNA cannot be used interchangeably because they operate through very different mechanisms, and thus the successful modification of an siRNA according to the teachings of Eckstein *et al.* is not predictable. Indeed, Eckstein *et al.* teach that while 2'-fluoro uridine does not considerably affect and/or abolish the ***catalytic properties*** of ribozymes, 2'-fluoro uridine "incorporated at specific positions of a ribozyme ***prevented cleavage at these positions by RNase A***," and, moreover, that "***protection by incorporation of a modified ribose sugar*** according to the present invention ***will be rather general and not be restricted to RNases*** which depend on the presence of the 2'-hydroxyl group" (column 2, lines 55-59). Accordingly, one of skill in the art would not have had the motivation, nor the expectation of success, based on the teachings of Eckstein *et al.*, to introduce 2'-fluoro nucleosides into an siRNA molecule, since the teachings of Eckstein *et al.* suggest that such a modification would prevent cleavage of the siRNA and thereby inhibit the ability of the siRNA to mediate RNAi activity. Based on the teachings of Eckstein *et al.*, one of skill in the art would not have expected that an siRNA comprising a 2'-fluoro nucleoside would be capable of mediating RNAi, let alone an siRNA comprising 2'-fluoro nucleosides at <u>each</u> cytidine and uridine in the antisense strand, as required by the instant claims.

Further, Eckstein *et al.* fail to teach or suggest ***the specific sugar-modified ribonucleotides of 2'-deoxyguanosine and 2'-deoxyadenosine,*** let alone the particular combinations and positions of these sugar-modified ribonucleotides within an siRNA, as required by claims 86-87. Indeed, Eckstein et al. ***teach away*** from the incorporation of 2'-deoxynucleosides into siRNA molecules. For example, Eckstein *et al.* teach that "the modifications according to the present invention are tolerated at least in certain positions, [while] in contrast, the incorporation of only 15% 2'-deoxynucleotides into a hammerhead ribozyme is reported to decrease the catalytic efficiency by two orders of magnitude," supporting the notion that "2'-fluoro analogs adopt a structure more similar to that of ribonucleotides than that of deoxyribonucleotides" (column 7, lines 13-17 and 22-25). Thus, one would not have been motivated, based upon the teachings of Eckstein et al., to incorporate 2'-deoxynucleosides into an siRNA, since Eckstein *et al.* teach that 2'-deoxynucleosides decrease activity of the RNA molecule into which they are introduced.

The teachings of Parrish *et al.* also fail to make up for the deficiencies of Tuschl *et al.* The teachings of Parrish *et al.* are generally directed to siRNAs. In particular, Parrish *et al.* teach modification at the 2' position of the nucleotide sugar in siRNAs, including 2'-fluoro uracil, 2'-aminouracil, 2'-aminocytidine, 2'-deoxythymidine and 2'-deoxycytidine, and that "2'-fluorouracil was compatible with RNAi activity." Parrish *et al.* fail to teach or suggest ***the specific sugar-modified ribonucleotides of 2'-fluoro cytidine, 2'-deoxy guanosine and 2'-deoxy adenosine,*** let alone the particular combinations and positions of these sugar-modified ribonucleotides within an siRNA, as required by the instant claims.

Moreover, Parrish et al. ***teach away*** from the claimed invention of claims 86-87. In particular, Parrish *et al.* teach that "[m]odification of cytidine to deoxycytidine (or uracil to thymidine) on either the sense or the antisense strand of the trigger was sufficient to produce a substantial decrease in intereference activity" and that, for this modification, "trigger activity was more sensitive to modification of the antisense strand than that of the sense strand." Thus, Parrish *et al.* teach that 2'-deoxy nucleosides are not well tolerated in siRNAs, and, in particular, are not well tolerated in the antisense strand. Accordingly, one would not have been motivated, nor had any expectation of success, based on the teachings of Parrish *et al.*, to substitute guanosine or adenosine in the ***antisense strand*** of an siRNA with a corresponding ***2'-deoxy***

nucleoside (*e.g.*, either at specific positions or throughout the entire antisense strand), let alone substitute ***each guanosine and each adenosine*** in the ***antisense strand*** of an siRNA (*e.g.*, either at specific positions or throughout the entire antisense strand).

In summary, the Examiner has failed to point to any teaching in the Tuschl *et al.*, Eckstein *et al.* and Parrish *et al.* references that would compel one of ordinary skill in the art to make the claimed invention. The prior art must suggest "to those of ordinary skill in the art that they ***should*** make the claimed composition or device, or carry out the claimed process" and ***[b]oth the suggestion*** and the ***reasonable expectation of success*** must be founded ***in the prior art, not in the applicant's disclosure*** (emphasis added)." *In re Dow Chemical Co.* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). In view of the foregoing, Applicants respectfully request that the rejection of claims 1, 3-4, 33, 38 and 84-90 under 35 U.S.C. §103(a) as being obvious over Tuschl *et al.* in view of Eckstein *et al.* and Parrish *et al.* be reconsidered and withdrawn.

CONCLUSION

In view of the above amendments and remarks, it is believed that this application is in condition for allowance. If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

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Respectfully submitted,

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